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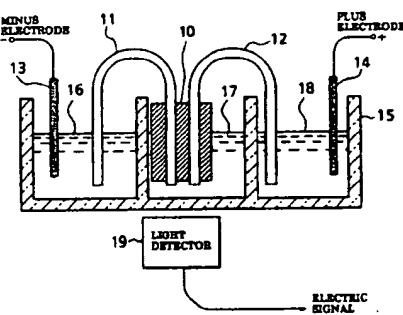
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(54) Electrophoresis apparatus of capillary type

(57) The electrophoresis apparatus of capillary type according to the invention has a capillary 11, 12 with gel for use in electrophoresis filled in a hollow portion thereof; a first buffer solution container 16 for storing a buffer solution and introducing a sample labelled with a fluorescent substance into an inlet of said capillary 11, 12 for electrophoresis; a second buffer solution container 17 for storing a buffer solution containing a luminescent solution, into which said sample is continually introduced from an outlet of said capillary 11 after electrophoresis; electrophoresis means 13, 14 for subjecting said sample to electrophoresis by applying a predetermined value of voltage to said gel through which said sample is being transferred while being electrophoresed; and light receiving means 19 disposed underneath the outlet of said capillary 11, 12 to read fluorescence emitted from said sample from bottom in a direction in which said sample is approaching closer. This electrophoresis apparatus has benefits that it can read an electrophoresis pattern of a sample of a nucleic acid or a protein obtained by subjecting the sample to electrophoresis with a high sensitivity without requiring any expensive device structure such as a laser beams source system, it is cheaper than conventional ones requiring such a laser beams system, and it is easy to handle.

Fig. 1



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labelled in the DNAs of the sample in the gel 31 is excited with the laser beams 40, thereby resulting in the emission of fluorescence 41 that in turn is received by the detector 37. The detector 37 fetches the received fluorescence 41 in a photomultiplier and the photomultiplier converts the fluorescence into electrical signals and transmits the electrical signals to the data processing unit 38. The data processing unit 38 is arranged such that the sequences of the fragments in the sample are determined by the molecular weights on the basis of the peak positions of the intensity of the fluorescence 41.

When they are employed as a sample, DNA fragments are labelled with the fluorescent substance so as to have different fluorescent wavelengths corresponding to their ingredients, and to determine the DNA sequences of the four bases simultaneously by only one fine tube. The fluorescent substance which can emit four different fluorescent wavelengths, may include, for example, fluorescein isothiocyanate (FITC), rhodamine isothiocyanate (EITC), tetramethylrhodamine isothiocyanate (TMRITC), and substituted rhodamine isothiocyanate (XRITC), respectively. Further, the gel electrophoresis apparatus of this type has a sensitivity to detection of DNAs in the order of 1×10^{-16} mole as high as the method using radioactive isotopes, when argon ion laser having wavelength of 488 nm or 514 nm is employed.

In addition, the above example briefly alludes to an example in which luminescence can be caused by taking advantage of chemical reaction energy. However, the example does not specifically disclose any procedures of the elution from a gel material, a reaction with a luminous substance, the removal of an unnecessary labelling substance, and the like. Actually, there are many specific problems to be solved in embodying techniques, which may include, for example, procedures for the supply of a source of chemically light-emitting energy, the mixture of the luminous substance with a labelling substance, and the removal of the labelling substance after luminescence in order to prevent background noises from being caused by residual substances.

The electrophoresis methods can be applied to, for example, the diagnosis of hereditary diseases, the investigation of DNAs in determining suspects of crimes and the investigation of the relationship between a parent and a child, in addition to the determination of the DNA sequence. In the diagnosis of hereditary diseases, it is currently possible to distinguish even one base from samples on the basis of the difference between the electrophoresis patterns by taking advantage of the difference in the structure of a high dimension under specific conditions (e.g. temperature or pH as causing a minute difference in denatured states of DNA) by the substitution of the base or bases inherent in each hereditary disease, such as single strand conformation polymorphism. The investigation of DNA in, for example, determining a suspect of crime and a parent-child relationship is made by comparing the difference in electrophoresis distances by

taking advantage of a deviation in DNAs (polymorphism) between individuals.

In such experiments, the base length of a DNA is approximately 1,000 bases or less in many cases, and the gel employed for electrophoresis is polyacrylamide gel. In the case where the base length is several thousands, agarose gel is employed. Further, a gel electrophoresis apparatus of a flat plate type is employed for the comparison of the electrophoresis pattern of a sample with a reference DNA electrophoresis pattern. With such an gel electrophoresis apparatus, the sample and the reference DNA sample are subjected to gel electrophoresis side by side for a ready reference to the difference between the two electrophoresis patterns.

These methods, however, require various cares in, for example, sustaining homogeneity of a gel with high stability and maintaining the uniformity of temperature on the electrophoresis plates during electrophoresis processes. In particular, a severe management of temperature using a thermostat is required in single strand conformation polymorphism. The management of temperature makes the cost of a device expensive and its size large because the electrophoresis apparatus of a flat plate type consumes a large amount of power and the amount of heat evolved is great. With respect to this point, the management can be performed easier by an electrophoresis apparatus of a capillary type because such apparatus can make its electrophoresis section smaller in size.

However, the prior art electrophoresis apparatus for reading the electrophoresis pattern obtained by the fluorescence detecting method of conventional technology requires the use of a laser light source of a unique type corresponding to the wavelength at which the fluorescent substance is to be excited. The conventional electrophoresis apparatus suffers from various disadvantages. Such a laser light source is so expensive and the cost of the laser light source, which amounts for the total cost of the apparatus, is so great that the cost of the apparatus itself becomes likewise expensive as well. Further, laser light emitted from the laser light source has a high energy density even if it would scatter, so that there is the risk of causing disorders or abnormality of vision, such as dyschromatopsia or blindness, if the laser light would enter the naked eye. Hence, such a laser light source should be handled with great care and should be incorporated in the electrophoresis apparatus with great attention paid to security from such laser light. This also leads to making the electrophoresis apparatus expensive.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an electrophoresis apparatus of capillary type which has such excellent characteristics that it can read an electrophoresis pattern obtained by subjecting nucleic acids or proteins to gel electrophoresis through a capillary with a high degree of sensitivity or resolution without requiring

for selectively gathering the fluorescence emitted from the electrophoresed sample in the vicinity of the outlet of the capillary. The light gathering device can reduce the fluorescence emitted from the sample remaining in an area where the fluorescence from the remainder of the sample exerts adverse influences upon a degree of sensitivity to detection and selectively gather the fluorescence in the vicinity of the outlet of the capillary, thereby detecting the fluorescence emitted from the sample with a high degree of sensitivity or resolution.

Still further, the electrophoresis apparatus of capillary type according to the present invention is provided with the first capillary having the gel for use in electrophoresis filled into a hollow portion thereof; the second capillary having the gel for use in discharging the sample by electrophoresis filled into a hollow portion thereof; the first buffer solution container in which the buffer solution is stored and which leads the sample labelled with the fluorescent substance to the inlet of the gel in the hollow portion of the first capillary; the second buffer solution container in which the buffer solution mixed with the light-emitting or luminous solution is stored and into which the electrophoresed sample is introduced continually from the outlet of the first capillary; the electrophoresis means for subjecting the sample to electrophoresis by applying a predetermined value of voltage to the gel contained in the hollow portions of the first capillary and the second capillary; and the light-receiving means disposed to read the fluorescence emitted from the sample introduced into the second buffer solution container from the bottom of the outlet of the capillary.

Furthermore, the electrophoresis apparatus of capillary type according to the present invention comprises a third buffer solution container into which the sample discharged from the second buffer solution container and transferred through the gel contained in the second capillary is introduced by application voltage thereto. Thus, the gel in the second capillary serves as transferring the sample from the inlet of the second capillary in the second buffer solution container toward the third buffer solution container, after the electrophoresis has been finished. In other words, the second capillary may constitute a passage through which the sample is transferred and discharged from the second buffer solution container, thereby causing the sample to be discharged gradually after the sample has been subjected to electrophoresis. As a consequence, this configuration of the electrophoresis apparatus of the capillary type allows the sample remaining in the second buffer solution container to be reduced and the fluorescence emitted from the remaining sample can be reduced, thereby lowering background noises and improving sensitivity or resolution to detection of the fluorescence of the sample to be received by the light receiving means.

The electrophoresis apparatus of capillary type according to the present invention may also utilize light from the light-emitting or luminous solution capable of generating chemiluminescence as light for activating or exciting the fluorescence emitted from the fluorescent

substance labelling the electrophoresed sample. For example, the such light-emitting or luminous solution may contain a luminous substrate that can produce an active intermediate substance emitting chemiluminescence. The employment of the light-emitting or luminous solution capable of generating chemiluminescence does not require laser beams and the optical energy of such an active intermediate substance may be absorbed by the fluorescent substance, thereby exerting a sufficient intensity of an activation or excitement action upon the fluorescent substance labelling the sample. As a result, the fluorescent substance of the sample is activated or excited to a sufficiently high level and generates fluorescence that in turn is detected by a light detecting device such as a photomultiplier, thereby providing an electrophoresis pattern having a satisfactory intensity of fluorescence and enabling the reading of the fluorescence at a sufficient degree of sensitivity and resolution.

The other objects, features and advantages of the present invention will become apparent in the course of the description that follows, with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

25 Fig. 1 is a block diagram showing an entire configuration of an electrophoresis apparatus of capillary type according to an embodiment of the present invention.

30 Fig. 2 is a view in section showing a light detecting device capable of providing an electrophoresis apparatus of capillary type with an improved degree of sensitivity according to a variant embodiment of the present invention.

35 Fig. 3A is a view showing a supply means for feeding a fresh supply of a luminous solution in accordance with a variant embodiment of the present invention. Fig. 3B is a view in section showing the supply means of Fig. 3A.

40 Fig. 4 is an abbreviated block diagram showing a brief structure of a conventional gel electrophoresis apparatus for a description of a method for the detection of a gel electrophoresis pattern using the fluorescence detection method.

45 Fig. 5 is a view showing details of part of a fluorescence detection section of the conventional gel electrophoresis apparatus for a description of a gel electrophoresis pattern using the fluorescence detection method.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention will be described in more detail by way of embodiments with reference to the accompanying drawings.

Fig. 1 is a block diagram showing an entire configuration of an electrophoresis apparatus of capillary type according to an embodiment of the present invention. In

5 container section 17, the buffer solution is mixed with the light-emitting or luminous solution containing the luminous substrate for exciting the fluorescent substance. The active intermediate substance of the luminous substrate contained in the light-emitting or luminous solution produces energy to excite the fluorescent substance of the sample, thereby causing the generation of fluorescence. The fluorescence emitted from the sample is detected by the light detector disposed underneath the second buffer solution container section 17, and is then converted into electrical signals.

Then, a description will be made of the operations of the electrophoresis apparatus of capillary type according to the present invention.

First, a sample containing DNAs is prepared and the DNAs are divided into fragments which are labelled with a fluorescent substance. The sample labelled with the fluorescent substance is then poured into the first capillary 11 that has been filled with gel for use in electrophoresis. The sample may be poured into the first capillary 11 directly through a syringe or indirectly by placing the first capillary 11 in a container containing the DNA fragments of the sample and applying voltage to the first capillary 11 to transfer the sample into the capillary on the basis of the principle of electrophoresis. After the sample has been transferred into the first capillary 11, it is then set to the electrophoresis apparatus as shown in Fig. 1, followed by the application of voltage in several kilovolts between the first electrode 13 and the second electrode 14 to start electrophoresis.

By the electrophoresis, the DNA fragments are moved through the first capillary toward the second buffer solution container section 17 because they have negative charges thereon in the buffer solution. As they reach the outlet side of the gel in the first capillary 11, the sample comes in touch with the luminous substrate of the luminous solution contained in the second buffer solution container section 17, and the active intermediate substance existing in the luminous substrate thereof provides energy to the fluorescent substance labelling the DNA fragments of the sample. As a result, the fluorescent substance generates fluorescence. The fluorescence emitted from the sample reaches the light detector 19 disposed under the second buffer solution container section 17, and the fluorescence detected by the light detector 19 is then converted into electrical signals.

In the electrophoresis apparatus of capillary type according to the present invention, the light detector 19 is disposed under the second buffer solution container section 17 and under the opening of the outlet of the first capillary 11. In other words, the light detector 19 looks toward the direction in which the sample is being discharged downward from the outlet of the first capillary 11 and approaching. More specifically, as shown in Fig. 1, the DNA fragments separated by electrophoresis flow downward from the outlet of the first capillary 11 into the buffer solution in the second buffer solution container section 17. Accordingly, the light detector 19 can detect fluorescence in the position immediately below the sample moving downwardly. This configuration has a merit that it can improve the efficiency of detection remarkably in comparison with the case where the detection is performed in the direction perpendicular to the sample moving direction.

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Once the sample has been transferred into the second buffer solution container section 17, a portion of the DNAs emitting fluorescence spreads into the buffer solution in the second buffer solution container section 17; 10 however, a majority of the sample is attracted by the electrophoresis voltage and transferred to the inlet side of the second capillary 12. The majority of the sample is transferred through the second capillary 12 to the third buffer solution container section 18. If the sample would spread 15 greatly around the outlet of the first capillary 11 into the buffer solution in the second buffer solution container section 17, such a great dispersion of the sample may be reduced to a large extent, for example, by forming gel of a low concentration around the opening ends of the 20 outlet of the first capillary 11 and the inlet of the second capillary 12 to allow the sample to move through such a gel from the outlet of the first capillary 11 to the inlet of the second capillary 12.

25

After the sample has been subjected to electrophoresis and its fluorescence emitted from the sample has been read, it is discharged from the second capillary 12 into the third buffer solution container section 18. When the sample reaches the inlet side of the second capillary 12 in the second buffer solution container section 17 with the aid of the action of electrophoresis, it is then pulled into the gel in the second capillary 12 from its inlet and transferred therethrough toward the outlet thereof and eventually into the buffer solution in the third buffer solution container section 18 by the action of electrophoresis. As a result, the sample is discharged from the second buffer solution container section 17. The sample may then be withdrawn from the third buffer solution container section 18 after the completion of electrophoresis. As this discharging system serves as removing 30 the sample from the second buffer solution container section 17, particularly from an area close to the outlet of the first capillary 12, the light detector 19 can efficiently receive and read the fluorescence from the sample with high accuracy around the outlet of the first capillary 11 35 immediately after the sample has been transferred from the outlet of the first capillary 11 into the second buffer solution container section 17 without undergoing adverse influences or interference from background noises that may otherwise be generated from the sample 40 remaining around the outlet thereof in the second buffer solution container section 17.

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Now, a description will turn to a variant of the electrophoresis according to the present invention, with reference to Fig. 2. This variant is directed to an electrophoresis apparatus of capillary type which selectively collects or gathers fluorescence emitted from a sample in order to improve a degree of sensitivity to detection.

sensitivity, even if the electrophoresis for a sequence of DNAs would be continued for a long period of time.

As described hereinabove, when the pigment such as ethidium homodimer, thiazole orange homodimer or oxazole yellow homodimer is employed, it is not required to label the sample with a fluorescent substance in advance. The such pigment is held between the double strands of the DNA in the stage that it has been eluted after electrophoresis and light is given out due to changes of luminous efficiency. Hence, it can likewise give out light merely when it is mixed with a buffer solution container containing a luminous substrate as described hereinabove.

As described hereinabove, the electrophoresis apparatus of capillary type according to the present invention can read an electrophoresis pattern in a stable manner without using an expensive and special device such as a laser beams source because the apparatus can excite a sample and cause the fluorescent substance of the sample to give out fluorescence by a reaction energy created by a chemical reaction. This electrophoresis apparatus can be manufactured at cheaper costs than conventional ones using expensive and special devices. Further, the electrophoresis apparatus can selectively and efficiently collect the fluorescence without undergoing any adverse influences from background noises by mounting a concave mirror around the outlet of the capillary in which electrophoresis is carried out. Furthermore, the electrophoresis apparatus of the capillary type is provided with the second capillary for discharging the sample after the completion of separation of the sample by electrophoresis, thereby enabling the sample to be discharged from the container in which the fluorescence is read soon after the reading of the fluorescence has been finished. This can greatly reduce an occurrence of background noises that may otherwise be caused by the sample remained in the container. This leads to the reading of an electrophoresis pattern with a high sensitivity and accuracy.

Claims

1. Electrophoresis apparatus of capillary type, comprising:
a capillary (11, 12) with gel for use in electrophoresis filled into a hollow portion thereof;
a first buffer solution container (16) for storing a buffer solution and introducing a sample labelled with a fluorescent substance into an inlet of said capillary for separation of said sample by electrophoresis;
a second buffer solution container (17) for storing a buffer solution containing a luminous solution, into which said sample is continually introduced from an outlet of said capillary (11) after separation of said sample by electrophoresis;
electrophoresis means (13, 14) for subjecting said sample to electrophoresis by applying a predetermined value of voltage to said gel through which

5 said sample is being transferred into said second buffer solution container (17) while being electrophoresed; and

10 light receiving means disposed underneath the outlet of said capillary (11, 12) to read fluorescence emitted from the fluorescent substance labelling said sample from bottom of the outlet of said capillary (11, 12) from which said sample is being discharged downward.

2. Electrophoresis apparatus according to claim 1, characterized in that said light receiving means is disposed underneath the outlet of said capillary (11, 12) disposed in said second buffer solution container (17) to read the fluorescence emitted from the fluorescent substance of said sample discharged downward into said second buffer solution container (17) in a direction in which said light receiving means faces an opening of the outlet thereof directed toward the bottom of the second buffer solution container (17).
3. Electrophoresis apparatus according to claim 1 or 2, characterized in that said light receiving means is provided with a light collecting device capable of selectively gathering the fluorescence emitted from the fluorescent substance of said sample in the vicinity of the outlet of said capillary (11, 12) in said second buffer solution container (17).
4. Electrophoresis apparatus according to at least one of claims 1 to 3, characterized in that said light collecting device is a concave mirror (27).
5. Electrophoresis apparatus according to at least one of claims 1 to 4, characterized in that said light collecting device is a combination of an oval-shaped concave mirror (27) with an iris (28).
6. Electrophoresis apparatus of capillary type, comprising:
a first capillary (11) with gel for use in electrophoresis of a sample filled in a hollow portion thereof;
45 a second capillary (12) with gel for use in discharging said sample by electrophoresis filled in a hollow portion thereof;
a first buffer solution container (16) for storing a buffer solution and introducing said sample labelled with a fluorescent substance into the gel poured in said first capillary (11) from an inlet thereof;
50 a second buffer solution container (17) for storing a buffer solution and introducing said sample separated through said first capillary (11) by electrophoresis thereinto from an outlet thereof;
a second buffer solution container (17) for storing a buffer solution and introducing said sample separated through said first capillary (11) by electrophoresis thereinto from an outlet thereof;
55 electrophoresis means (13, 14) for subjecting said sample to electrophoresis to the gel filled in said first capillary (11) and said second capillary (12) by

Fig. 1

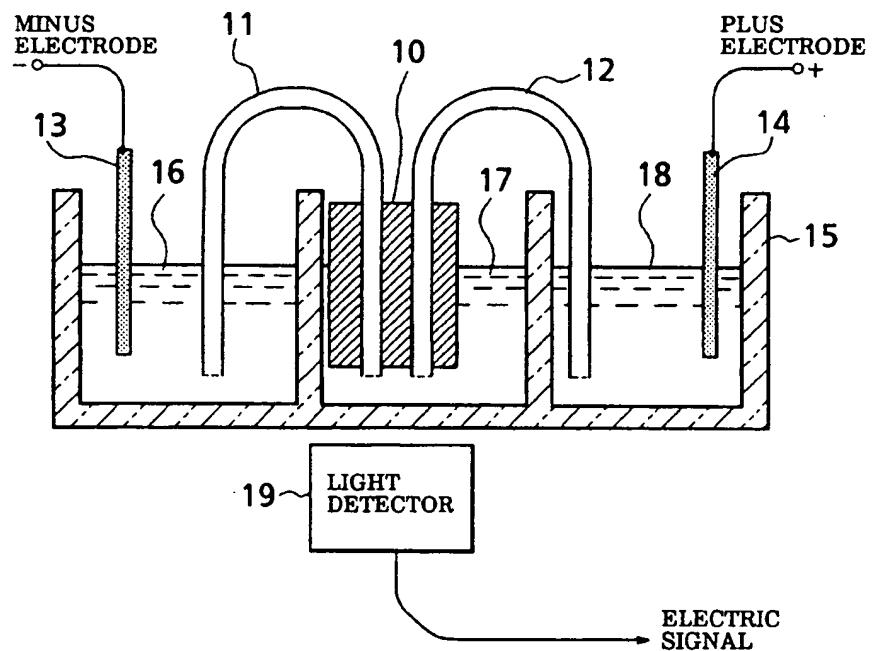


Fig. 3A

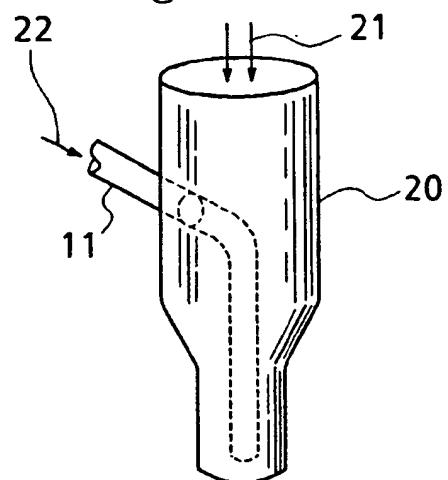
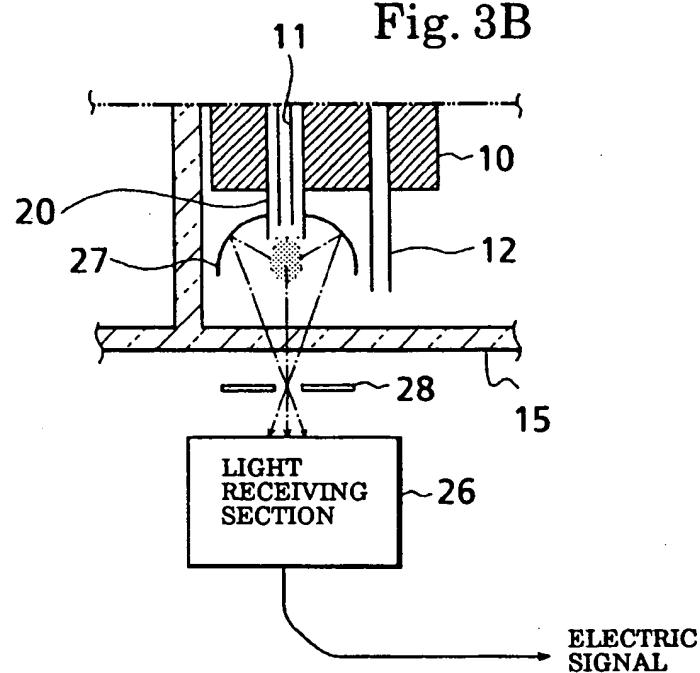
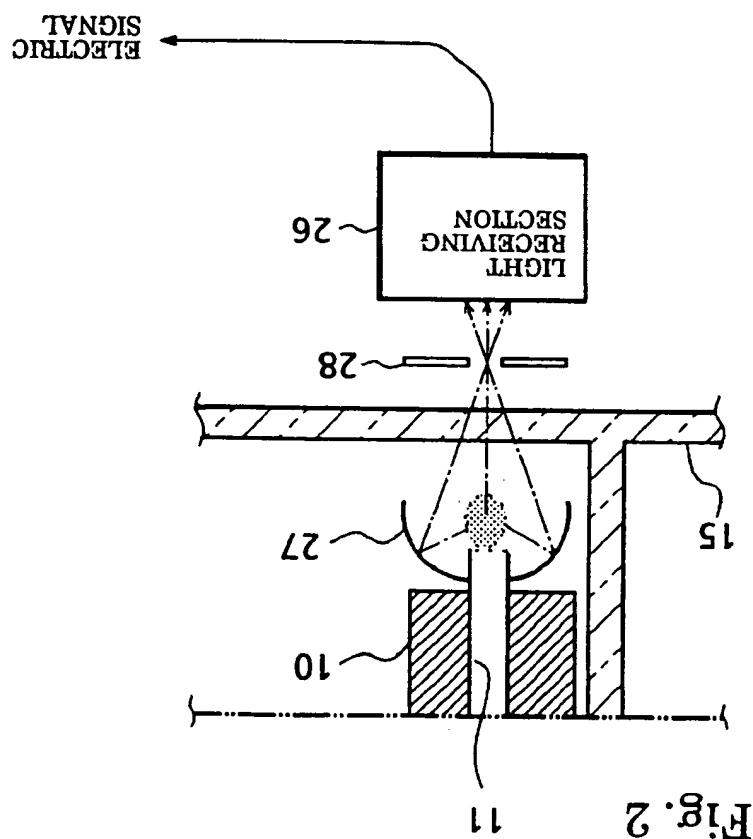


Fig. 3B





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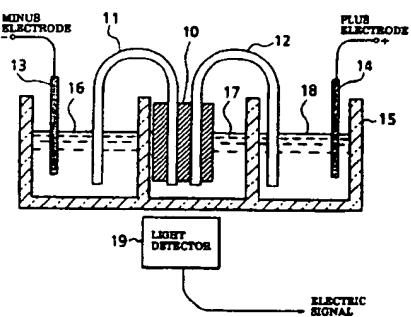
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(54) Electrophoresis apparatus of capillary type

(57) The electrophoresis apparatus of capillary type according to the invention has a capillary 11, 12 with gel for use in electrophoresis filled in a hollow portion thereof; a first buffer solution container 16 for storing a buffer solution and introducing a sample labelled with a fluorescent substance into an inlet of said capillary 11, 12 for electrophoresis; a second buffer solution container 17 for storing a buffer solution containing a luminescent solution, into which said sample is continually introduced from an outlet of said capillary 11 after electrophoresis; electrophoresis means 13, 14 for subjecting said sample to electrophoresis by applying a predetermined value of voltage to said gel through which said sample is being transferred while being electrophoresed; and light receiving means 19 disposed underneath the outlet of said capillary 11, 12 to read fluorescence emitted from said sample from bottom in a direction in which said sample is approaching closer. This electrophoresis apparatus has benefits that it can read an electrophoresis pattern of a sample of a nucleic acid or a protein obtained by subjecting the sample to electrophoresis with a high sensitivity without requiring any expensive device structure such as a laser beams source system, it is cheaper than conventional ones requiring such a laser beams system, and it is easy to handle.

Fig. 1



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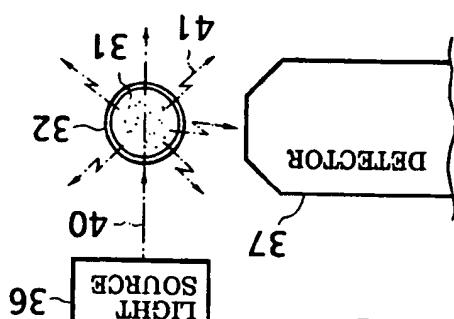


Fig. 5

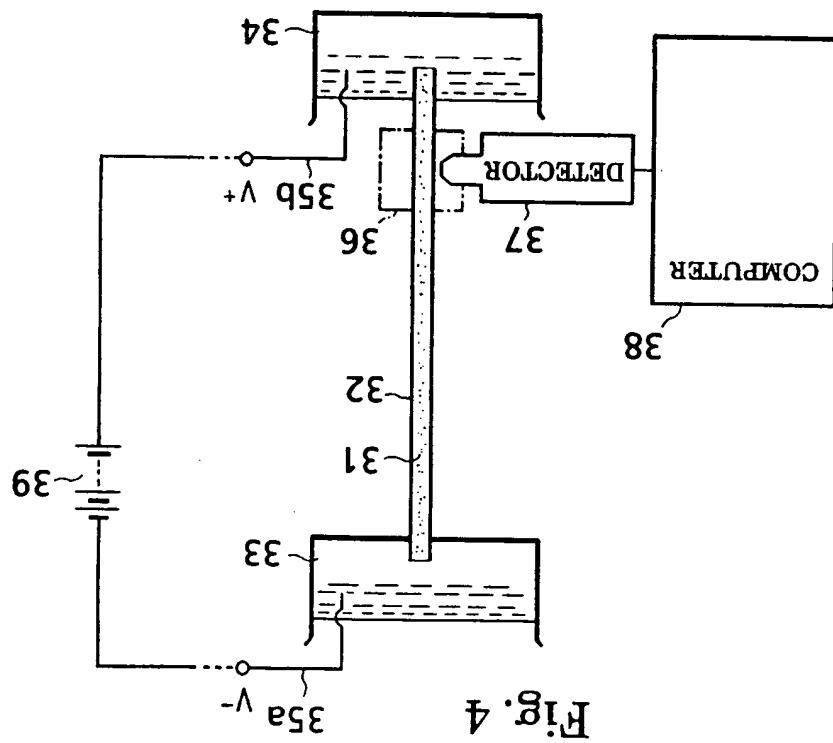


Fig. 4